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Development of a Humanized Mouse Model for Direct Evaluation of Anti-Human PD-L1 Antibodies

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INTRODUCTION

Therapies that perturb binding of the programmed death-ligand 1 (PD-L1) to its receptor, programmed cell death protein 1 (PD-1), have achieved unprecedented rates of sustained clinical response in patients with various cancer types. Mouse surrogate antibodies were initially evaluated in syngeneic mouse models as a proof of concept for testing the efficacy of anti-PD-L1 therapies. However, there is an urgent need to develop appropriate animal models to directly evaluate anti-human PD-L1 antibodies before they reach clinical trials. Here we describe our ongoing efforts for the development of a chimeric mouse/human cell line to test human anti-PD-L1 antibodies. PD-L1 expression was profiled by FACS using an anti-mouse PD-L1 antibody, which confirmed expression of PD-L1 in a series of murine cancer cell lines. H22, a liver cancer line with a moderate expression level of PD-L1 was selected due to its *in vivo* sensitivity towards anti-PD-1 and anti-PD-L1 agents. The CRISPR/Cas9 system was employed to knockout the murine PD-L1 and replace it with the human counterpart. Targeted knockout was confirmed by sequencing, while expression of human PD-L1 was detected by FACS using an antibody that specifically recognizes human PD-L1 and does not cross-react with the mouse isoform. The engineered H22-hPD-L1 cell line was inoculated subcutaneously into immunocompetent BALB/c mice to establish an *in vivo* model with a fully competent murine immune system, harboring a humanized PD-L1. Tumor growth for the H22-hPD-L1 model was followed over time and its sensitivity to anti-human PD-L1 antibody was established.

METHODS

SgRNA/Cas-9 mediated cleavage of mPD-L1 and introduction of hPD-L1

sgRNA cloning ➔ Transfection of target cells with Cas9/gRNA ➔ Stable clone selection and expansion ➔ Evaluation of mPD-L1 expression level by FACS ➔ Extraction of genomic DNA ➔ Sequencing to confirm disruption of mPD-L1 ➔ Infection with lentivirus expressing hPD-L1 ➔ Confirmation of hPD-L1 expression level by FACS.

Flow cytometry

Cells re-suspended in staining buffer (PBS +3% FBS) were stained with: PE anti-mouse CD274 (PD-L1) (Biolegend, 124308); PE rat IgG2b, κ isotype control antibody (Biolegend, 400608); PE anti-human CD274 (PD-L1) (Biolegend, 329706), or PE mouse IgG2b, κ isotype control (Biolegend, 400314). Stained cells were subsequently washed in staining buffer twice and then analysed with FACS Caliber (Becton Dickinson, San Jose, CA).

Western blotting:

Murine cell lysates were subjected to SDS PAGE followed by western blot with Mouse B7-H1 MAb (Clone 179711), Rat IgG2A (R&D Systems, MAB1019) at a dilution of 1:500.

***In vivo* efficacy evaluation**

8-10 weeks female Balb/c mice were subcutaneously inoculated with 5 x10^6 humanized H22 cells. Treatment started 11 days post cell inoculation by i.p. injection of 5 mg/kg human anti-PD-L1 antibody or isotype control. Tumor volume was measured twice a week.

RESULTS

Figure 1. Analysis of mPD-L1 expression by western blot.

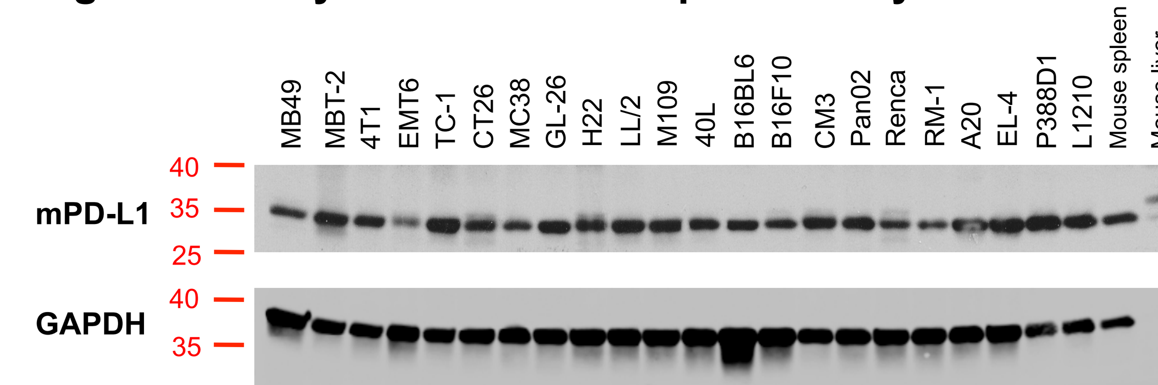


Figure 2. Representative mPD-L1 antibody staining in 4 cell lines.

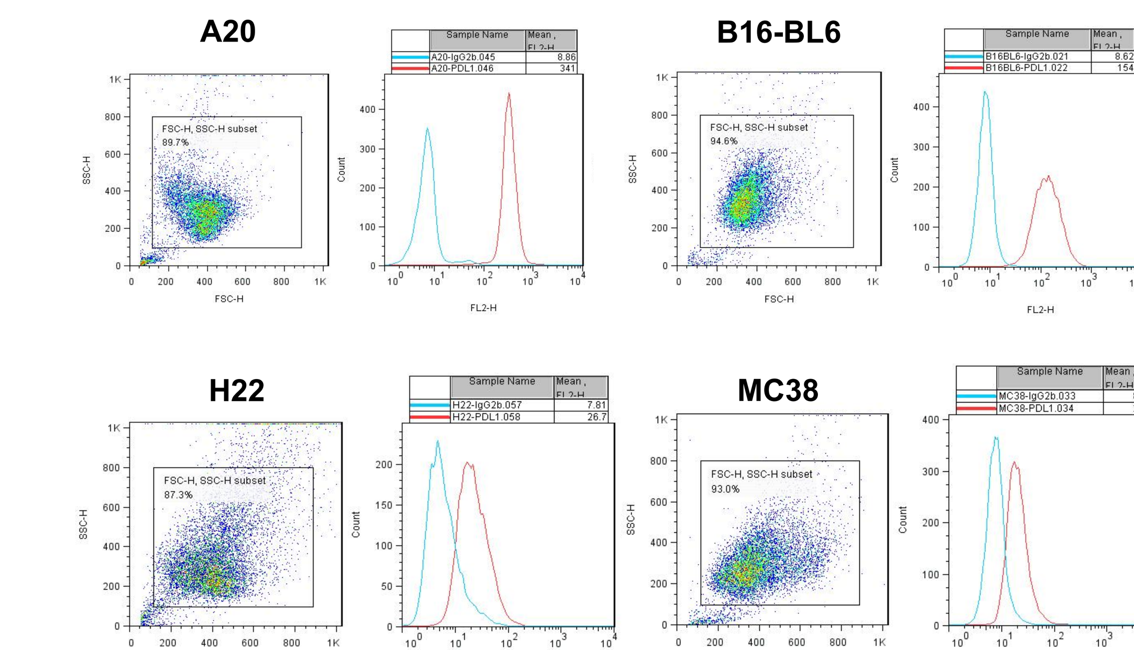


Figure 4: m-PD-L1 knockout in the H22 cell line. Comparison between endogenous mPD-L1 expression in H22 (parental) and several mPD-L1 null clones derived from the H22 cell line (A); sequencing data showing disruption of mPD-L1 in a selected clone (B).

Table 1. List of murine cell lines for comparison of mPD-L1 expression by western blotting and FACS.

Cancer type		Cell line
Solid tumors	Bladder	MB49
		MBT-2
	Breast	4T1
		EMT6
	Colon	CT26
		MC38
	Glioma	GL-26
	Kidney	Renca
	Liver	H22
	Lung	LL/2
	Melanoma	B16BL6
		B16F10
		CM3
	Pancreatic	Pan02
	Prostate	RM-1
Blood malignancies	Leukemia	L1210
		P388D1
	Lymphoma	A20
		EL-4

Figure 3: Schematic illustration of the genetic engineering strategy.

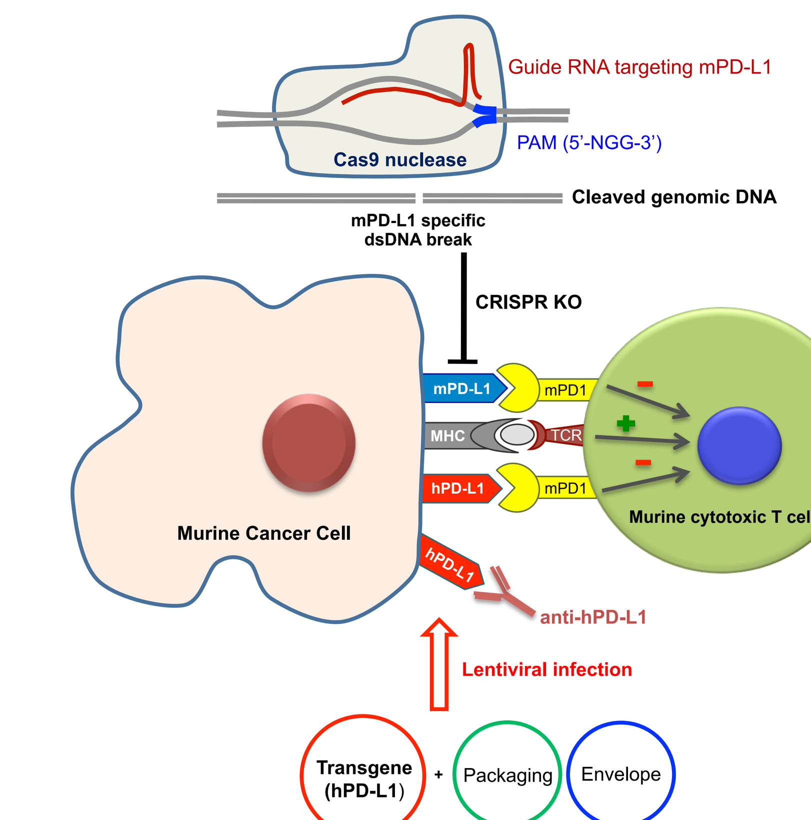


Figure 6: Response to anti hPD-L1 treatment in the humanized PD-L1 H22 model. Anti-hPD-L1 antibody inhibits tumor growth. Animal No = 8; Tumor growth inhibition (TGI) = 58%.

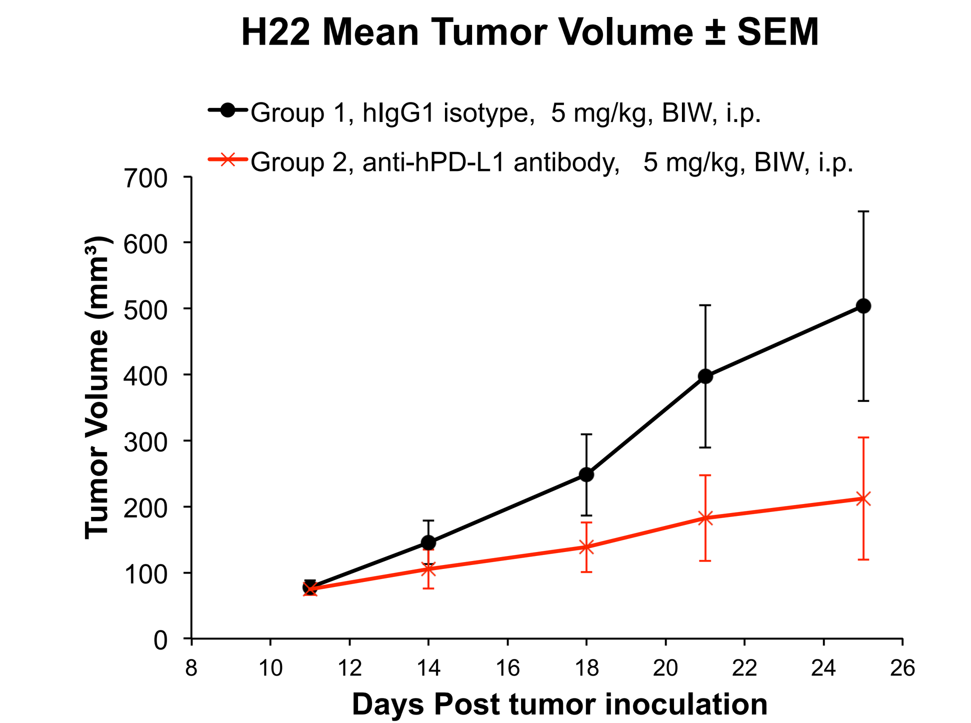
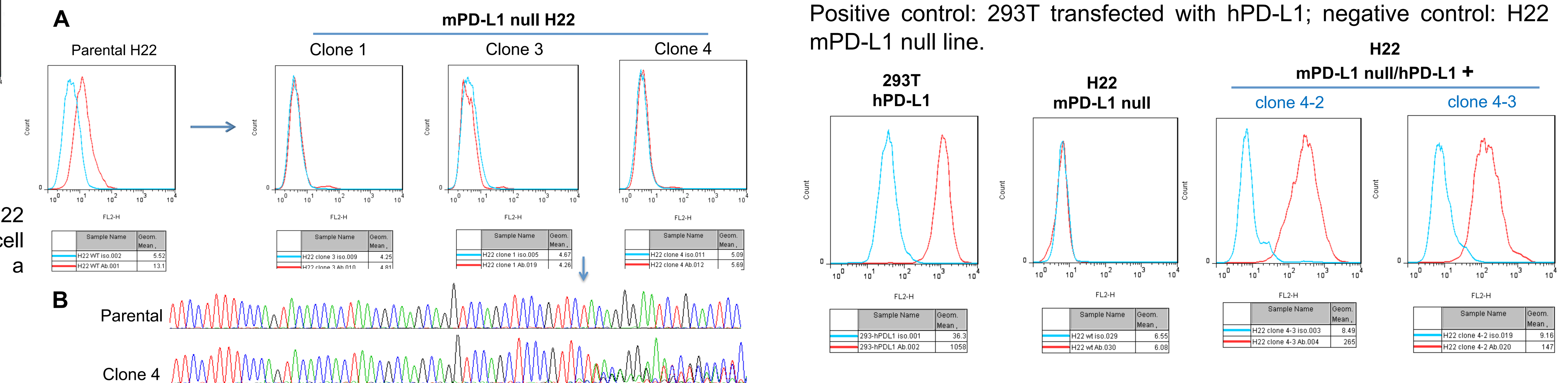


Figure 5: Confirmation of hPD-L1 expression by FACS Analysis. The mPD-L1 null H22 cell line was engineered to express hPD-L1. Positive control: 293T transfected with hPD-L1; negative control: H22 mPD-L1 null line.



SUMMARY

In summary, our H22-hPD-L1 model is a valuable tool to evaluate the *in vivo* activity of anti-human PD-L1 antibody therapies either as single agents or as combination strategies. Similar engineering may be applied to more murine cell lines to provide a panel of cell lines from different diseases and various genetic makeup to test immunotherapies involving anti-hPD-L1 antibodies.